ANNUAL PROGRESS REPORT

THERMAL INJURY OF THE SKIN

November 15, 1958-May 1, 1960

By: University of Illinois
Chicago, Illinois

To: Office of Naval Research
Department of the Navy
Physiology Branch (Code 441)
Washington 25, D. C.

Grant No. NR 114 161

Principal investigator

Sol Roy Rosenthal, M.D., Ph.D.

In Cooperation with

Graham P. Lewis, Ph.D.

Assisted by

Alan B. Goodman
Wilma A. Spurrier
Donald Findlay
Frank Higgins

INTRODUCTION

During the period of this report (1959-60) the opportunity arose to apply the principle of the "toxin-antitoxin" phenomenon in burned or injured animals, as described in previous ONR reports (1954-58), to human subjects.

This undertaking was on an emergency basis precipitated by the tragedy of the Our Lady of the Angels School fire on December 1, 1958. The vastness of the project necessitated the cooperation of many groups, all acting as a "team" in an attempt to establish basic principles and practical applications. The cooperation of these groups was initiated and pursued by the Chicago Branch of the Office of Naval Research by its Chief Scientist, Dr. A. R. Dawe. The following were the cooperating agencies and their major contributions:

1. St. Anne's Hospital (Dr. James Hartney and Dr. Thomas Hartney)
   Administration of the healed burned donor blood or plasma to the burned or injured children.

2. Naval Medical Research Unit No. 4 (Dr. L. F. Miller, M. J. Rosenbaum, R. I. Lytle, and B. Sullivan)
   Tissue culture and electrophoretic studies of the blood of the burned and injured subjects.

3. University of Notre Dame (Dr. Thomas G. Ward and Louise I. Lindholm)
   To test the hypothesis of the role of infection in thermal death.

4. University of Illinois and Cook County Hospital (Dr. Sol Roy Rosenthal, Wilma A. Spurrier, and Alan B. Goodman)
   Serology of the blood of the burned or injured subjects from St. Anne's and Cook County Hospitals; coordinating of the various scientific studies.

The overall study is described in the following pages. Individual and more specific studies will be published by the cooperating groups. Abstracts of papers as they appear in the Federation Proceedings and presented at the annual meeting of the American Societies for Experimental Biology (April 11-15, 1960) in Chicago, Illinois, follow the detailed presentation.

~~In summarizing,~~ "toxins" were demonstrated in the blood of acutely burned or injured human subjects as determined by the cytotoxic effect on HeLa cells, the cytolytic effect on red cells of acutely burned or injured individuals, and precipitinogens against healed burn sera.

"Antitoxic-antibody"-like substances were demonstrated in the sera of healed burned individuals by their neutralization of the cytotoxic effect on HeLa cells and the presence of "hemolysins" and precipitins.

In preliminary clinical trials it was found that the administration of blood or plasma from healed burned donors to critically toxic burned or injured subjects was associated with a precipitous change in their clinical condition which was manifested by increasing awareness, appetite, and spontaneous mobility, and a decrease in local edema, pain, irritability, and restlessness. The post-transfusion sera of such critically ill subjects lost their tissue culture toxicity which corresponded with the clinical improvement.

Further clinical trials are recommended using "titred" antisera of known in vitro potency.

Further evidence that the cause of late thermal death is not basically one of infection was demonstrated in germfree animals where it was shown that the death rate was higher following a standard thermal insult in germfree animals than in normal ones.

The opinions expressed in the following pages--"The 'Toxin-Antitoxin' Phenomena in Burned and Injured Human Subjects"--are not to be construed as representing the official position of the United States Navy.

THE "TOXIN-ANTITOXIN"* PHENOMENA IN BURNED AND INJURED HUMAN SUBJECTS

The existence of a specific "toxin" following burning has generally been denied (1, 2, 3, 4). Davis and Abbott (2), in a review of the subject (1956), state: "Although the existence of a toxin has not been completely ruled out, none has been found to date and a careful study of the clinical and laboratory data usually reveals the cause of death."

The majority opinion is that soon after burning there is loss of fluid, colloids, and electrolytes. If the burn is extensive and severe, there results a diminished blood volume, a diminished cardiac output, and finally a collapse of blood pressure with accompanying toxic manifestations and death. The late toxic manifestations (after two days) and death usually are explained by secondary infection.

Experimental (dogs--Gilmore [5]; and monkeys--Lasker and Fox [6]) and clinical studies carried out to correct the fluid, colloid, and electrolyte loss, plus antibiotics and chemotherapeutic agents to counteract infection, have resulted in only a slight improvement of the mortality following severe burns. In a study at the Massachusetts General Hospital (7) on the mortality rate in 785 cases of burns admitted to the hospital, it was found that during the period 1939 to 1942, when the method of treatment was more or less primitive, the mortality rate was 15.6% (of 133 cases). During the period between 1943 and 1954 when there were adequate intravenous therapy, transfusions, antibiotics, aseptic care, attention to nutrition, and early excision and grafting, there was only a 5% decline in the mortality rate. When the latter period is further broken down to differentiate changes in antibiotics and newer forms of therapy, it was found that between 1943 and 1947 the

---

* The term "toxin" is used in its broadest sense, i.e., any substance or group of substances that produce non-physiological effects on cells *in vitro* or *in vivo*. Similarly, the term "antitoxin" is used broadly to include any counteraction *in vivo* or *in vitro* to these "toxins."

mortality rate was 10.7% (of 248 cases) and from 1948 to 1954, 10.4% (of 355 cases). The mortality rate was found to be related directly to the severity and extent of the burn. Younger individuals (0 to 15 years old) and older persons (55 and over) were less resistant than the middle-aged groups. The sharp rise in the mortality rate began with 30% or more of body surface burns (second and third degree). Similar results were obtained at the Brooke General Hospital (8) where, likewise, the most modern methods of treatment were employed plus the fact that therapy was instigated promptly. When approximately 50% of the body surface is involved in severe burns, the mortality rate is approximately 50%.

Since the mortality following severe burns remains high, notwithstanding replacement therapy, aseptic technique, and antibiotics, other factors causing this mortality must be considered. One of these is that as a result of the degradation products resulting from burning of the skin and other tissues, a specific "toxin", or "toxins", is produced which enters the circulation and contributes to the toxic manifestations and death of the individual. In 1937 Rosenthal described the presence of a "toxin" in the blood of burned shoats, adult pigs, guinea pigs, and humans (9). An "antitoxin" likewise was described in the blood of healed burned pigs and humans (10).

In order to isolate this "toxin" free of blood, a direct in vivo method was developed in rats to obtain diffusates from burned skin circumventing the blood (11). This material proved to be toxic and lethal to mice and rats either in acute or chronic experiments (12). When injected into rabbits, precipitins were produced against this "toxin" and agglutinins and "hemolysins" against the red cells of recently burned rats. In a preliminary experiment the rabbit rat-"toxin" antisera protected against rat "toxin" in mice. The demonstration of a "toxin" followed by autoimmunization likewise was detected by serological means in the blood of burned rats and mice (13).

At this stage of the experiments with the demonstration of burn "toxin-antitoxin" in animals and humans, the Our Lady of the Angels School fire occurred in Chicago (December 1, 1958). Dr. Rosenthal presented his results to the medical staff at St. Anne's Hospital and suggested that blood be collected from individuals who had been burned by various means or who had had extensive injuries, but in whom these injuries now were completely healed, and that such whole blood or plasma be transfused into the burned victims at regular intervals (14). The medical staff at St. Anne's Hospital elected to do this for selected severely burned patients under their care. The problem now was to obtain sufficient healed burned donor blood as certain criteria had to be met--the donors had to meet the usual standards of the American Blood Bank Association and some arbitrary classification had to be set up for defining suitable donors. The following categories were established:

Category 4: 25% or greater body area burn within one year

Category 3: 50% or greater body area burn within two to five years

Category 2: 50% or greater body area burn within six to ten years

Category 1: 50% or greater body area burn eleven years or longer

By a publicity campaign in the Chicago area and elsewhere (14), a limited amount of blood was obtained to meet the above requirements.

No attempt will be made in this report to consider the management of these burned or injured patients either locally or generally. Many of the children sustained fractures, contusions, and abrasions as a result of jumping from upper stories. This phase of the treatment will be reported by the staff of St. Anne's Hospital. All that will be considered here are such studies pertaining to the "toxin-antitoxin" phenomena in burned human subjects. Another series of burned patients from the Cook County Hospital were studied serologically over periods of 0 to 128 days following injury.

Plan of Procedure

The major premise of the school fire undertaking was to help the critically ill children by the use of blood or plasma from healed burned donors. All other considerations were incidental and at no time were laboratory specimens taken expressly for "study." Use was made of blood specimens after the normal routine laboratory procedures had been completed. Blood or plasma was administered according to the private doctors' orders, but, when available, instead of using routine blood donors, healed burned blood donors were substituted.

The clinical subjective findings before and after transfusions were noted by two doctors and recorded. The routine temperature, pulse, and respiration were carried out by the nursing staff as usual. Urine and blood studies were performed as indicated in critically burned subjects.

In both St. Anne's and Cook County Hospital patients the presence of "toxin" in the blood was determined by one or more of the following tests--cytotoxic effect on HeLa cells, cytolytic effect on red blood cells, and presence of precipitinogens to healed burn sera. The appearance of "antitoxins" and/or "antibodies" was detected in the sera of convalescent and healed burned individuals by their anticytotoxic effect on HeLa cells and the appearance of "hemolysins" and precipitins.

Demonstration of "Toxins" (Chicago School Fire--St. Anne's Hospital Patients).

1. "Cytotoxins" (HeLa Cells).--The sera of acutely burned individuals, when tested as is routine for determining the presence of virus on 48- to 72-hour monolayers or sheets of mature cells, had little or no effect. However, when a suspension of HeLa cells in Eagle's Basal Media was incubated with 0.2 ml. of sera from extensively burned or injured individuals, practically no growth occurred after 72 to 96 hours. The exact method and detailed results have been reported elsewhere (15). Table I gives the results of some of the

TABLE I

DETERMINATION OF "TOXINS" IN BLOOD OF BURNED OR INJURED HUMAN SUBJECTS

Age	% Burn	Fracture	"Cytolysins" (RBC)				"Cytotoxins" (HeLa)	
			Time Drawn (Days)	(1)	(2)	(3)	Time Drawn (Days)	Absent or Present
9	27	-	0	-	-	-	1	+
13	40	-	0	-	-	-	14	+
9	46	+	0	4*	2	-	13	+
12	19	-	1	-	-	-	2	+
13	25	-	1	-	-	-	1	+
13	44	+	1	-	-	-	1	+
13	53	-	1	8	8	8	1	+
9	63	-	1	16	4	8	9	+
9	23	H	2	-	-	-	2	-**
8	55	-	9	8	8	2	2	+
13	61	-	9	4	4	8	1	+
14	-	MF	1	-	-	-	2	+
10	-	MF	1	-	-	-	1	+
10	-	MF	1	-	-	-	1	+
13	-	MF	9	8	4	8	1	+

(1) Patient's sera + patient's RBC + complement
(2) Patient's sera + other burned patient's RBC + complement
(3) Patient's RBC + healed burn sera

* - Titre or dilution of sera
** - With hemogenate of RBC inhibition occurred

MF - Multiple fractures
H - Hemorrhages

\+ Present
\- Negative

severely burned and/or injured children in the school fire. In four cases there were no second degree burns but multiple fractures plus abrasions and contusions. Fourteen of the 15 sera inhibited HeLa cell growth. The one exception was a child who had a 23% body burn plus internal thoracic hemorrhage. However, homogenizing his red cells by sonic vibrations and testing this material proved inhibitory to HeLa cells. These sera were tested for their cytotoxic effect on HeLa cells about 13 to 14 days after they were drawn. However, the sera of four of the critically ill drawn 11 to 16 days after injury and tested within a few days likewise were cytotoxic. As little as 0.05 ml. of the pooled toxic sera had this effect (fig. 1). For controls, sera from febrile (15 cases) or asymptomatic (12 cases) normal young adult males were not found to be inhibitory. To simulate storage conditions of the burn sera, aliquot specimens obtained from the 12 asymptomatic cases without previous history of burns or trauma were allowed to remain with clot for 13 days at $4^{\circ}C$. At the end of this period these sera did not inhibit HeLa cell growth.

2. "Cytolysins"* (RBC).--No agglutinins or "hemolysins" were detected when patients' red cells were added to healed burned donor sera as was the case in animal experimentation. However, when patients' cells were added to healed burned donor sera or to patients' sera plus complement (guinea pig) and incubated for 12 to 24 hours at $37^{\circ}C$., hemolysis but not agglutination was found. The exact method and detailed results will be published elsewhere (16). In the six burned or injured children on the critical list, "cytolysins" were found. No hemolysis occurred without the addition of complement or when normal red cells or sera were used. It will be noted from Table I that the sensitivity under these conditions was not as great as with the HeLa cell technique. These sera and red cells were tested one to two weeks after drawing. As will be discussed below, with sera and cells tested shortly after drawing and 48 hours or more after burning, the sensitivity was greater. The sera of

* The term "cytolysin" refers to any substance or substances causing lysis of red cells.

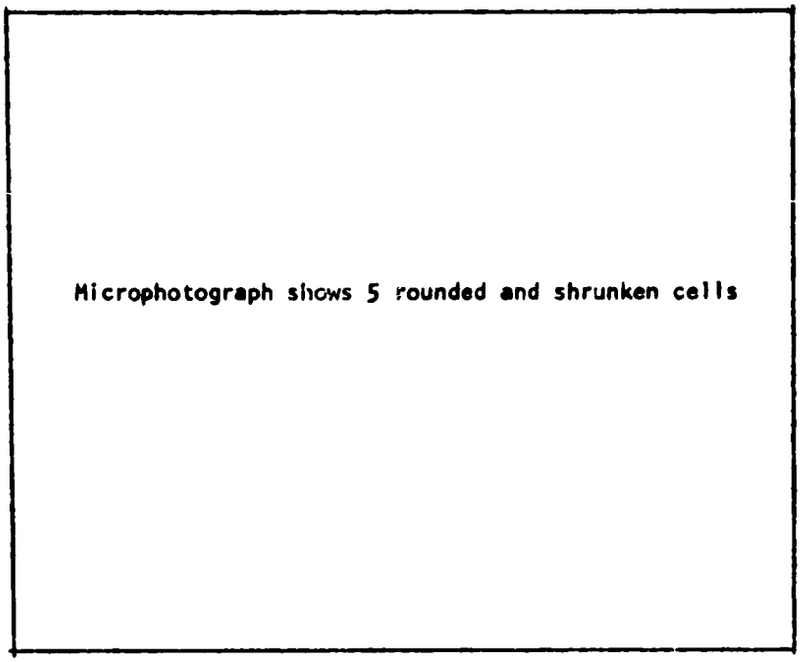


Fig. 1.--HeLa cell culture (72 hours) grown in 0.05 ml. pooled acute burn sera plus 0.2 ml. pooled normal sera.

Note rare rounded cell indicative of no growth.

(X 600)

non-burned individuals (33 cases) tested for "hemolysins" with red cells of acutely burned or normal individuals were all negative.

Demonstration of "Antitoxins."

1. HeLa Cells.--In four critically ill subjects one or two tests of their sera before transfusion with healed burn sera were found to be inhibitory to HeLa cell growth (fig. 2), whereas after transfusion (immediately or several days later) failed to inhibit HeLa cell growth (fig. 3). In a non-critical case where no cytotoxicity was noted, shortly after debridement and reduction of a wrist fracture, serum cytotoxicity appeared. In another instance (non-critical) where cytotoxicity was noted, it disappeared spontaneously only to reappear with clinical toxicity. In vitro tests incubating aliquots of toxic sera plus aliquots of healed burn sera (categories 4 and 3) and determining the effect of this combination on the growth of HeLa cells demonstrated a neutralization of the cytotoxic effect of the toxic sera (fig. 4) present in a degree not present in sera of subjects without a previous injury or burn (23 normal subjects tested). The neutralizing effect was found to the same degree in the gamma globulin fraction of healed burn sera (category 4) and not in the gamma globulin of individuals without a history of previous injury or burn.

2. "Hemolysins."--Healed burned donor sera (categories 4 and 3), when tested with red cells of burned individuals plus complement, gave hemolysis in five of the six on the critical list. The negative result was obtained in a child whose blood was obtained the same day of the burn. The sera of non-burned or injured individuals did not have this property (33 cases).

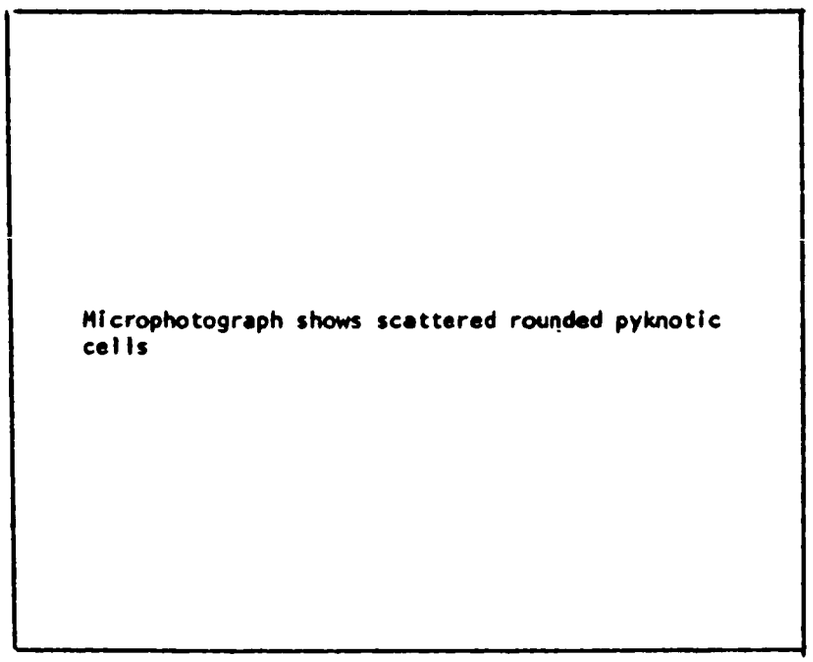


Fig. 2.--HeLa cell culture (72 hours) grown in 0.2 ml. acute burn sera (14 days after burn and before transfusion).

Note few rounded cells indicative of no growth.

(X 200) (Case 2 of text)

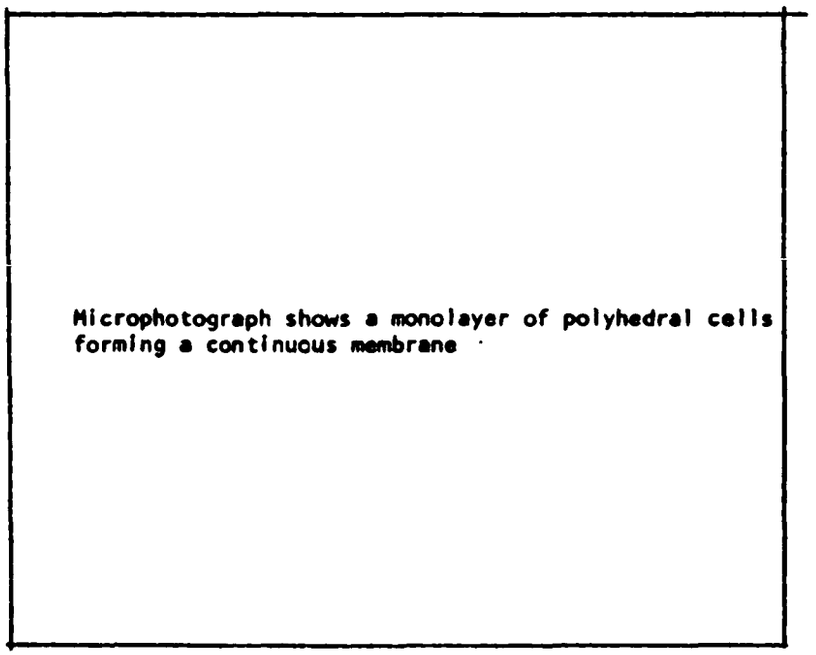


Fig. 3.--HeLa cell culture (72 hours) grown in 0.2 ml. acute burn sera (15 days after burn) from same patient as in fig. 2 but several hours after transfusion of healed burn sera.

Note continuous monolayer of polyhedral cells indicative of maximum growth (4+).

(X 200)

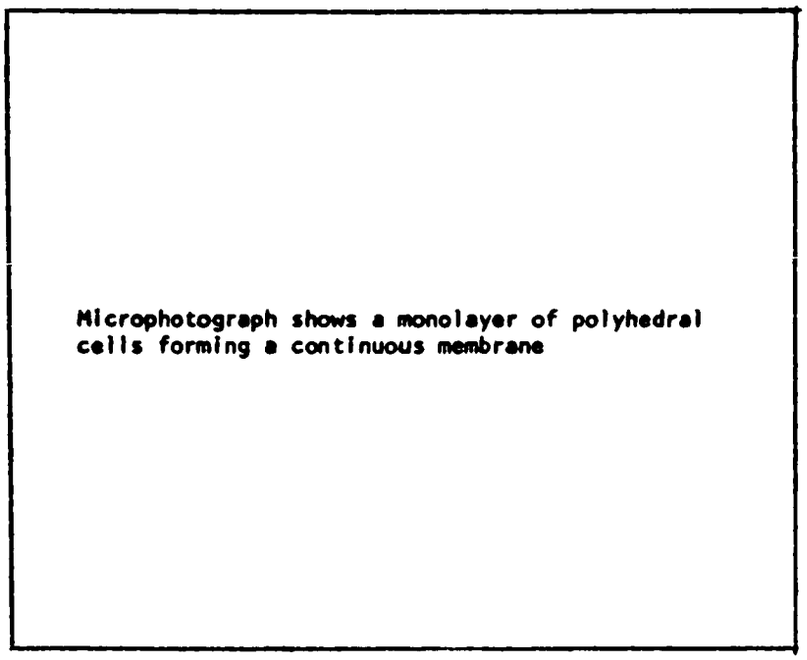


Fig. 4.--HeLa cell culture (72 hours) grown in 0.05 ml. pooled acute burn sera (same as in fig. 1) plus 0.2 ml. pooled healed burned donor sera.

Note monolayer of polyhedral cells (3+ to 4+ growth).

(X 600)

Time of Appearance of "Toxin" and "Antitoxin" in Burned Individuals (Cook County Hospital Patients).

According to a definite design of study, blood was drawn from burned patients soon after entering the hospital and at weekly and then bi-weekly intervals where possible.

Twenty-four burned subjects varying in age from 14 months to 48 years and sustaining second and third degree burns from 2-65% of body surface were studied (Table 2). In eight subjects, two or more bleedings were made available. In six subjects who sustained 5-65% of body surface burn and in whom blood was drawn one to 24 hours after injury, none developed detectable "cytolysins" or precipitinogens (ring test). In nine subjects with 2-28% body surface burn in whom blood was obtained 25 to 48 hours after burning, two (7% and 28% body burn) were positive (22%). In eight subjects with 5-65% body burn tested from 49 hours to six days following injury, six were positive to one or both tests (75%). A 4% burn tested 51 hours after the injury was positive to both tests. During the period seven to 35 days following burning (4-25% body burn), nine subjects in whom 44 tests were run were all negative for "toxins" ("cytolysins" and precipitinogens) and "antibodies" (precipitins and/or "hemolysins"). "Antibodies" were detected in the two subjects in whom blood was drawn from 40 to 135 days after burning (eight tests).

Clinical Results

Because of the limited burned donor blood available, especially Rh negative blood, only a limited number of transfusions with healed burn blood or plasma were given. The results to be considered thus will be confined to short periods of time before and after a series of such transfusions. Clear-cut results were seen when at least 500 ml. of whole blood or 250 ml. of plasma were given in a 24-hour period. Six critically ill children received this amount of blood or plasma 14 to 29 days after the fire. The clinical impression was that

TABLE 2

TIME OF APPEARANCE OF "TOXIN-ANTITOXIN" IN BURNED HUMAN SUBJECTS

Time	Extent of Burn (%)	"Cytolysins"				Precipitinogens-Precipitins			
		No. Cases	No. Cases Positive	No. Tests	No. Tests Positive	No. Cases	No. Cases Positive	No. Tests	No. Tests Positive
0-24 hr.	5-65	6	0	6	0	6	0	6	0
25-48 hr.	2-28	9	2	11	4	8	1	8	1
49 hr.-6 da.	5-65	8	6	15	11	6	5	6	5
7-35 da.	4-25	9	0	28	0	8	0	16	0
40-135 da.	-65	2	2	8	4	2	2	4	4

there was an improvement in all; in some it was described that a "crisis" had occurred within hours after the transfusion (in three subjects who received more than minimal amount of blood or plasma). Although new antibiotics were constantly being tried and must be considered in evaluating the clinical effects of the healed burn sera, the fact that the antibiotics were never the same in each subject and that in every case the clinical effects occurred within hours after the administration of the healed burn sera speaks for a specific effect of the sera.

The case histories in the three patients who received three to six transfusions within a six-day period (i.e., more than the minimal) are given below:

CASE 1: Nine-year-old female; extent of burn--46%, and fracture.

Condition of child before burned donor transfusions (December 14, 1958).--Child had received up to this point six units of regular blood bank plasma and five units of blood distributed on a daily basis. On December 12 she received one unit of blood and on December 13, two units of plasma. Child was markedly restless and irritable; any change of position would audibly exaggerate the irritability. Interest in her environment was slight; appetite poor; slept only in snatches; movements of extremities limited and painful; appeared extremely toxic; face, neck, and extremities (site of burns) markedly edematous; temperature--101 to 105 F (December 13 and 14).

Transfusion with 400 ml. burned donor blood begun December 14 at 7:10 p.m.

Condition of child on December 15 at 11:00 a.m.--Nurse and parents were not aware that type of blood of transfusion was different from those given previously. The changes listed below were noted by the parents, the nurse, and two physicians who had seen the child before and after the transfusion.

A marked and sudden change for the better was noted. Shortly after the transfusion the child fell asleep and slept for five hours--this was the first time she had really slept for days. Her irritability was so reversed that she began to dictate letters for her friends to her father. Her appetite was markedly improved, she had less pain, and the spontaneous mobility of her extremities was definitely increased.

On December 15 she received no transfusions of any kind. That night she slept about five hours.

On the morning of December 16 the child was holding her gains well. Toward noon, and increasing with evening, she again became restless and irritable, her appetite slackened, her mobility decreased, and the parents were notably disturbed and discouraged. At 8:50 p.m. 150 ml. of burned blood donor plasma was started and at 10:15 the infusion was complete. Soon after the transfusion the child fell asleep and slept all night--this was the longest time she had slept during her illness. At 10:00 a.m. on December 17 the impression of parents, nurse, and/or two physicians was as follows--

The child was better than she had ever been; more alert; more spontaneous movements; less pain, good appetite. What was particularly striking was that the face and neck, which had been markedly swollen, had receded to such an extent that they were of normal size. A decrease in the swelling had been suspected for the past two days. Temperature--101.8 F (rectal).

December 17--Transfusion 250 ml. of burned donor plasma.

December 18--All gains hold; child ate a fair breakfast of cereal and juice; drinking well; not so difficult a nursing problem; parents in relatively good cheer; neck and face edema still down.

December 18--Transfused with 250 ml. burned donor blood.

December 19--Transfused with 250 ml. burned donor blood and two units blood bank plasma.

December 20--Transfused with 250 ml. normal donor whole blood and 150 ml. burned donor plasma.

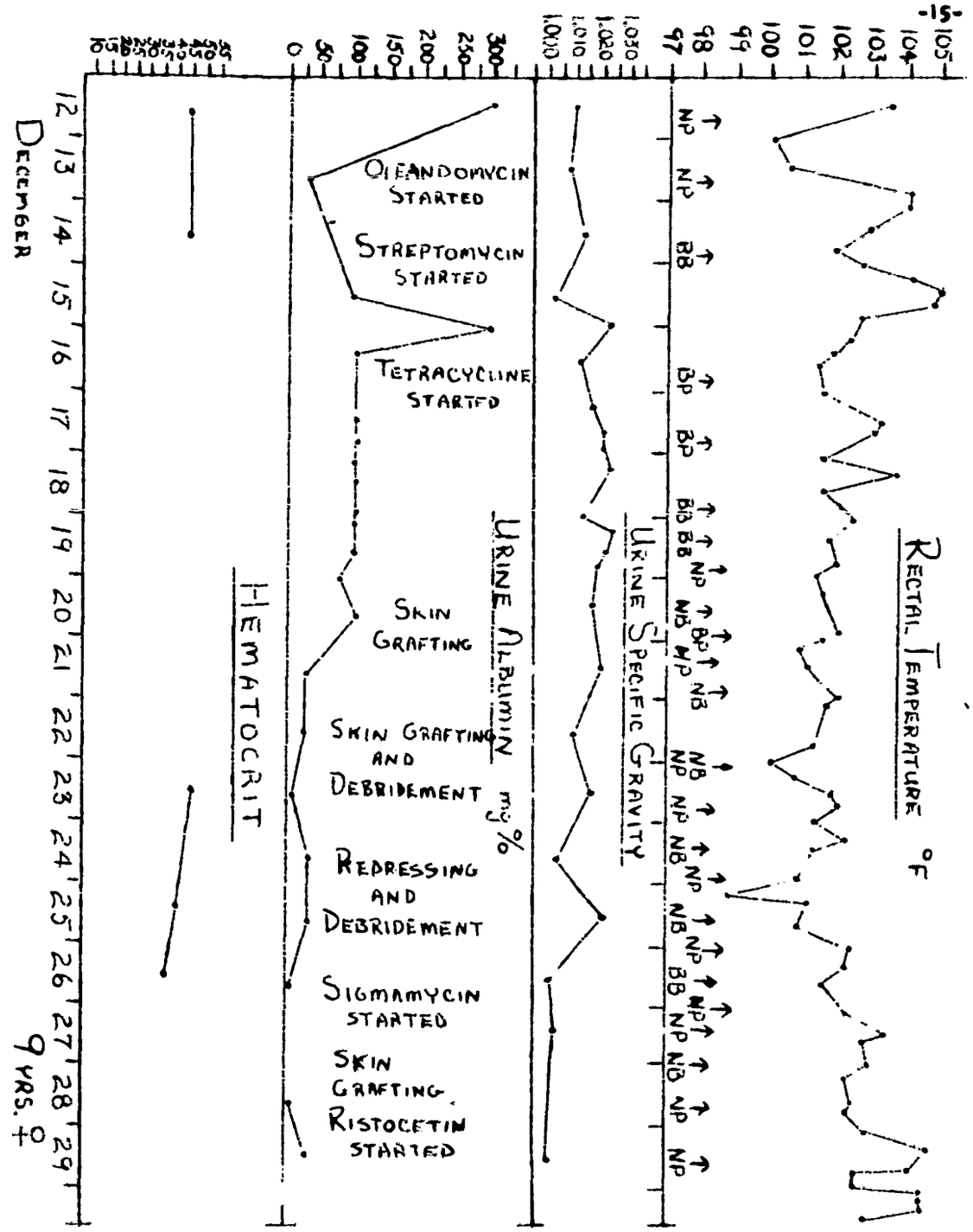
December 26--Transfused with 250 ml. burned donor blood and 250 ml. blood bank plasma. Child doing well; face--no edema (only exposed part); child has been skin-grafted; mother and father in good cheer (a good sign).

The temperature and selected clinical laboratory studies are plotted in Graph 1. After transfusion with burned donor blood and plasma from December 14 to 20 there was a fall in temperature and urine albumin. Three new antibiotics were initiated from December 13 to 16.

CASE 2: Thirteen-year-old male; extent of burn--61%.

Condition of child before burned donor transfusions (December 14, 1958).--Child had received up to this point nine units of whole blood and seven units of plasma from the blood bank. The blood or plasma was given almost daily, the last bloods being on December 11 and 12. At this time he was highly toxic; in a semi-confused state; restless; appetite poor; and slept fitfully. Spontaneous motion of his arms was limited. Edges of wounds of chest were black with green purulent discharge. Spiking temperature--100 to 106.8 F (December 11). He was transfused with 150 ml. burned donor blood at night (December 14) and with 250 ml. the following morning.

Condition of child on December 15, 11:30 a.m.--Grandmother who had been with the child daily stated on her own volition, not knowing that he had received any special type of blood, that there had been a crisis Monday morning, December 15. He was more interested in his surroundings; he asked about people at home; there was less pain; he slept better; his appetite improved; and he began to have more spontaneous movements. While two of the doctors were talking to the grandmother, the nurse came out of the patient's room and signalled to them to enter. The patient had just received his second bottle of blood and



Graph 1 (Case 1): Temperature and selected clinical laboratory determinations
NP = normal plasma BP = burned donor plasma
NB = normal blood BB = burned donor blood
Arrow indicates approximate time of administration.

was "leading the band" on the radio with his arm; he had not been able to move that arm before. Temperature--101 F.

On December 16 (noon) he showed no regression; was alert; slept frequently; appetite good; and oral and fluid intake considered good. There was a certain amount of fatigue. Temperature--101.8 F. From 9:40 to 11:10 that evening he received 250 ml. burned donor plasma.

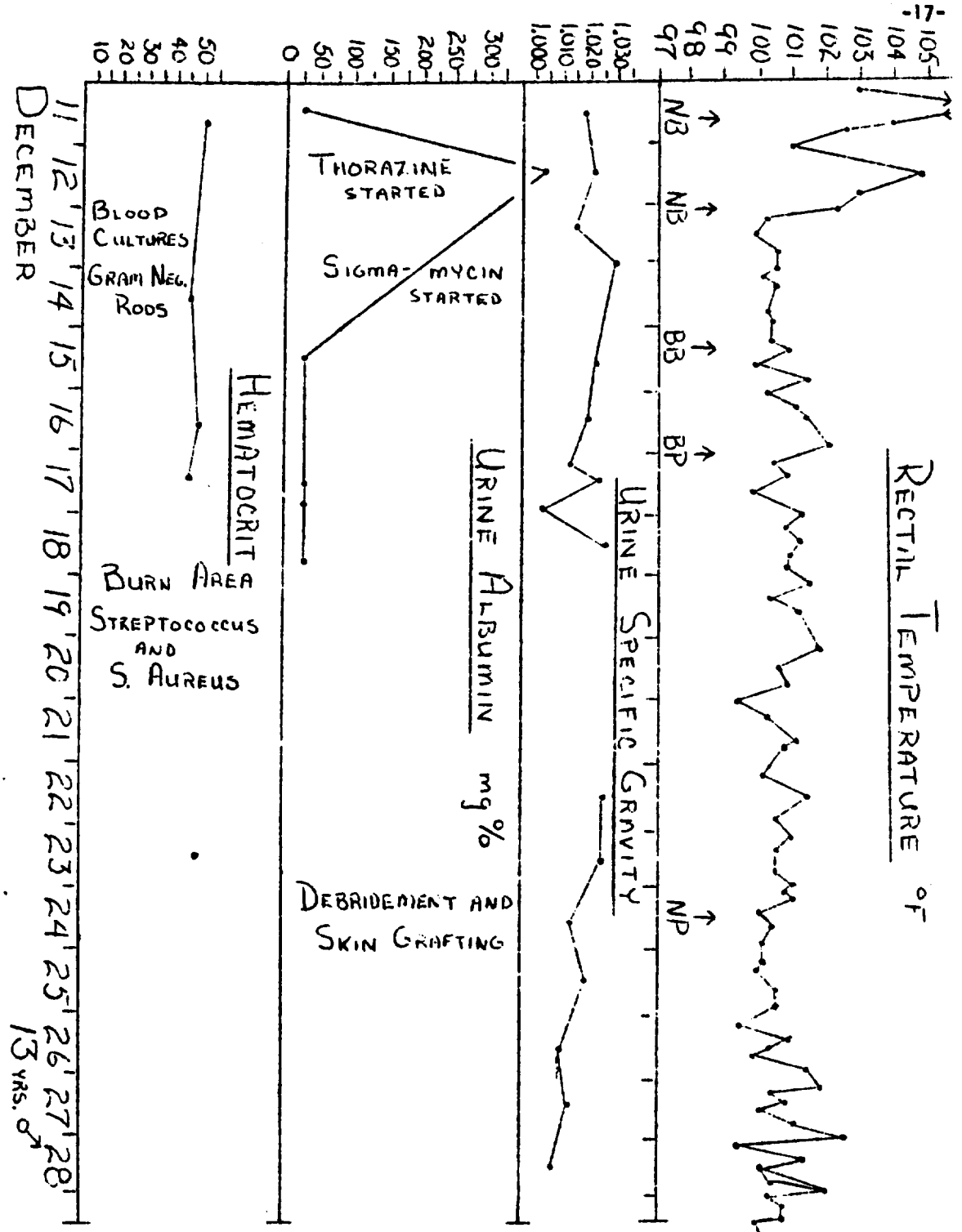
December 17--Child was as bright, alert, and aware of surroundings as he had ever been (two doctors' and nurse's impression). His appetite was good, he slept well, and the nurse observed that he perspired for the first time (the room was warm). Temperature--99.8 to 101 F. The wounds seemed to have changed appreciably. On December 16 the wound edges oozed a greenish purulent material (chest), but on December 17 the discharge was more serous and drier and generally healthier looking--this was noted by the doctor and the nurse.

December 18--Eating and drinking well; moves well; doctors, nurses, and relatives agree that improvement continuing.

In Graph 2 is shown that the temperature, which had been spiking from 101 to 106 F, began to fall off on December 12 and 13 before burned donor blood was given; however, after giving this blood and plasma, this lower level of temperature was maintained. The specific gravity of the urine and the urine albumin fell to lower levels after the burned donor transfusions. Two new antibiotics were started just before the above transfusions.

CASE 3: Thirteen-year-old female; extent of burn--53%.

Condition of child before burned donor transfusions (December 16).--Child received almost daily transfusions of a total of six units of whole blood and 18 units of plasma up to December 12. On December 15 she developed signs and symptoms of pneumonia and meningitis, with twitchings, convulsive movements,



Graph 2 (Case 2): Temperature and selected clinical laboratory determinations
NP = normal plasma BP = burned donor plasma
NB = normal blood BB = burned donor blood
Arrow indicates approximate time of administration.

etc. The parents were informed that the child's condition appeared hopeless. On December 16 she was semi-comatose; respirations stertorous; responded only to painful stimuli or loud command. Temperature--101 to 103 F (rectal). On December 16 (8:30 to 10:00 p.m.) she received 250 ml. burned donor plasma.

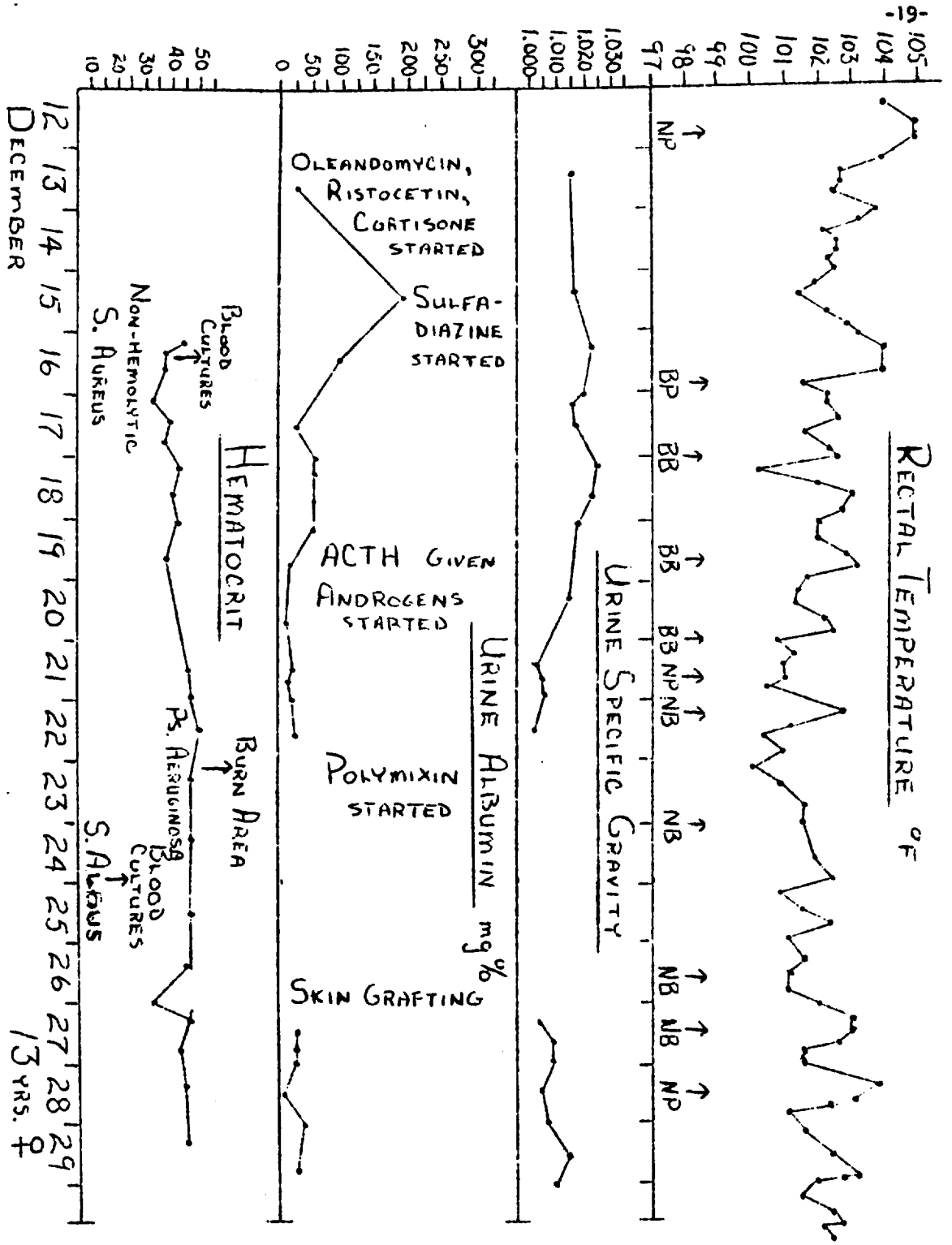
December 17, 8:50 a.m.--Relatively quiet; response to command perhaps slightly better than yesterday. 5:45 p.m.--Nurse and mother both state that child could take solid food (Jello) for the first time and fluids without gagging. Less irritable; movements somewhat improved. 10:00 p.m.--received 250 ml. of burned donor blood.

Decmeber 18, 8:30 a.m.--Improvement marked. Child opened eyes fully for first time and actually focused on held-up fingers and could tell how many fingers up. Speech clearer; respiration clearer and more regular (less rhonci); took fluids and solids without choking. Doctors, nurses, and parents enthusiastic--they felt a remarkable change for the better had taken place. 9:15 p.m.--sleeping naturally; respiration regular and not labored. Ate solids well today. Temperature--100 to 101.2 F.

December 19 and 20--250 ml. burned donor blood.

December 24--Two attending men to the child attested that something akin to a "crisis" occurred around the sixteenth to nineteenth of December. This child, who had been considered hopeless, was now alert and eating and sleeping well; she was well aware of her surroundings and conversed freely.

In Graph 3 it will be noted that there was a fall in temperature corresponding to the burned donor transfusions. The specific gravity of the urine and its albumin content also tended to fall. Two new antibiotics and cortisone were started just before the burned donor transfusions. Blood cultures were positive before and after the administration of the antibiotics and cortisone.



Graph 3 (Case 3): Temperature and selected clinical laboratory determinations
NP = normal plasma BP = burned donor plasma
NB = normal blood BB = burned donor blood
Arrow indicates approximate time of administration.

Discussion

There are undoubtedly numerous factors which contribute to the toxic and lethal effects following burning. The fluid, colloid, and electrolyte loss; nervous, hormonal, nutritional disturbances; and secondary infection are important factors. Cognizance of these factors unquestionably has diminished the mortality following thermal injury of moderate severity, but the death rate following severe and extensive burns still remains high (7, 8).

The diffidence of accepting the existence of a "burn toxin" rests to a great extent on the inability of many workers to detect the presence of a "toxin" in the blood of burned individuals or animals. The usual method for determining the presence or absence of a "toxin" is to inject the blood or plasma of burned animals into normal ones. The results at best were equivocal. Under given conditions, however, the blood of burned animals, when injected into previously burned ones, results in death of the animals (9). It is known that the blood of normal individuals or animals can detoxify many types of toxins. This property has been demonstrated for the properdin-complement system (17) and the endotoxin detoxifying component (EDC)oof the blood (18). These substances have been shown to detoxify the action of bacteria (gram negative) or their toxins and certain viruses, but are not known to increase after immunization. It is possible that these or other substances normally present in the blood may to a certain extent neutralize the "burn toxin", whereas the reticulo-endothelial system may actually remove toxins, bacteria, etc. (19).

Rosenthal and his group (11, 12, 13) have been able to isolate a "toxin" directly from diffusates of burned skin of rats in vivo circumventing the circulation. This "toxin" is fatal to mice or rats in acute or chronic experiments. Injection of this "toxin" into rabbits produces precipitins to the "toxin" and hemoagglutinins and "hemolysins" to red cells of acutely

burned rats. In preliminary experiments in mice, this rat-"toxin" rabbit antisera, when incubated with or injected after rat "toxin", detoxifies appreciably the action of the "toxin."

The human studies here reported support the animal work. Thus far it has been shown that the serum of burned human subjects is cytotoxic to HeLa cells, cytolytic to injured red cells, and that the sera of healed burned humans have an enhanced capacity to neutralize the cytotoxic effect on HeLa cells in vitro, and clinical evidence suggests a similar in vivo effect. The latter effect was associated with clinical improvement.

From the results here reported and from previous experimental evidence it is postulated that following injury, tissue destruction follows as a result of the injury proper. This process in turn releases or activates many enzyme systems that continue the further degradation of the tissue (20). These degradation products enter the blood stream (11) and can be toxic or lethal to the host (12). They also can act as antigens stimulating autoimmunization in the host (13).

It has been shown in animals by immunologic methods that many types of injury (thermal, radiation, or chemical) produce toxic and lethal degradation products which call forth immune bodies which are similar but not identical (21).

The time of detection of cytotoxic and cytolytic agents in the blood following burning or trauma differed in these studies. One or the other of the "toxins" appeared shortly after the injury and remained for varying periods dependent upon the extent of the injury. The role of the normally occurring detoxifying agents in the blood, absorption of "toxin" by red cells, reticuloendothelial system, etc., is yet to be determined.

Clinical combined with laboratory evidence suggests that sera of healed burned individuals may act as an "antitoxin" when injected into acutely

and severely burned or injured individuals. This form of therapy was first recommended by Rosenthal in 1937 and has recently gained support from Russian sources (22).

The detection of "antitoxins" in the blood of burned individuals here tested was not until 40 days after the injury when healing was well on its way. It is likely that antibodies appear sooner but in the presence of an open wound with degradation products continually forming, neutralization of antibodies present in the blood probably occurred and thus they excaped detection. It is of interest in this regard that in severe burns the catabolic process is known to continue for long periods of time (30 days or more) as demonstrated by a negative nitrogen phosphorus and potassium balance (23).

"Antitoxins" and/or "antibodies" ("anticytotoxins", "hemolysins", and precipitins) were detected in healed burned donors within five years after burning (categories 4 and 3). The fact that these properties were found in convalescent or healed burn sera and not in normal sera and were contained almost entirely in the gamma globulin fraction ("anticytotoxins") speaks for true antibody formation.

The total effect of healed burned donor blood in acute burns is yet to be determined. The clinical results here reported should be considered as preliminary only since the availability of blood was limited, the onset of treatment was delayed (14 to 29 days), laboratory methods had to be developed, etc. Clinically there was improvement in the six cases who received the minimal amount of blood or plasma. In the three who received more than the minimal amount the improvement often was described as a "crisis." The improvement was characterized by a decrease of pain, irritability, and edema, and an increase in awareness, mobility, appetite, and ability to swallow and sleep. It is true that in some instances a new antibiotic was started but they differed in each child and the clinical effect followed within hours after the administra-

tion of the healed burn sera. The clinical improvement was accompanied by a drop in the temperature and a decrease in the urinary output of albumin consistent with a decrease in toxicity. In some instances a decrease in the specific gravity of the urine was noted which would be expected with receding edema. In four of the critically ill where blood was available for testing before and after transfusion with healed burned donor blood or plasma, an *in vivo* neutralizing effect on HeLa "cytotoxin" present before transfusion was suggested. This phenomenon, likewise, often paralleled the clinical improvement. It is believed that not all sera of burned individuals have enhanced detoxifying properties. The three tests here presented (HeLa tissue culture, "hemolysins", and precipitins) may serve to "titre" the blood for potency.

The contribution of infection to the late mortality in severe and extensive burns is undoubtedly great. In one report (24) where 1,000 burn cases were treated by the most modern methods including antibiotics and chemotherapeutic agents, the mortality rate from septicemia accounted for 55% of all deaths. Yet a high percentage of the bacterial strains isolated from the blood were sensitive to one or more antibiotic.

One possible explanation for the above discrepancy is that toxins of various origins are known to injure the reticulo-endothelial system and render such hosts infinitely more susceptible to injury or infection (25, 26). In this study "cytotoxins", "cytolysins", and precipitinogens were demonstrated almost immediately or shortly after burning before any serious infection was noted. It is believed that the lowered resistance established by the degradation products of the injured tissue makes such individuals highly susceptible to organisms not infrequently present on the skin, in the environment, or in the nose, pharynx, or hands of personnel ministering to the patients.

It is proposed that amelioration of the basic injury caused by the "burn toxins" will be necessary before the host can cope efficiently with bacteria or other noxious agents that invade it. On the basis of presently

available data, it is believed that one method worthy of further investigation to effect such an amelioration is by the use of healed burned donor sera of known *in vitro* antitoxic potency. The clinical protocol for such investigations should include daily transfusions of blood (250 ml.) or plasma (125 ml.) from healed donors whenever transfusions of blood or plasma normally would be indicated or when toxicity is present.

Summary

1. Evidence for the demonstration of a "toxin" in the blood of acutely burned or injured human subjects as determined by three different methods has been presented; they are the cytotoxic effect on HeLa cells, the cytolytic effect on red cells of acutely burned or injured individuals, and precipitinogens against healed burn sera.

2. The presence of an antitoxic-like substance in the blood of healed burned individuals to the "burn toxin" has been demonstrated. Three distinct tests for "antitoxin" are presented--neutralization of the cytotoxic effect on HeLa cells, "hemolysins", and precipitins.

3. Preliminary clinical evidence suggests a beneficial and detoxifying effect of healed burn blood or plasma in critically burned or injured individuals.

4. More clinical trials are recommended using "titred" antisera of known *in vitro* potency.

REFERENCES

1. Harkins, N. H.: The Treatment of Burns, Charles C. Thomas, Springfield, Illinois, 1942.

2. Davis, J. H., and Abbott, W. E.: The Pathology of Thermal Burns--Changing Concepts, Ann. Surg., 40:788, 1956.

3. Boer, Karl A.: Pathology and Physiology of Burns (1942-1951), U. S. Armed Forces Medical Library Reference Division, Washington, D. C., (June) 1952.

4. Symposium on Burns (Nov. 2-4, 1950), National Research Council, Committee on Surgery, Subcommittee on Burns, Washington, D. C., 1951.

5. Gilmore, J. P., and Fozzard, H. A.: Hepatic Function Following Thermal Injury, Fed. Proc., 17:53, 1958 (No. 208).

6. Lasker, S. E., and Fox, Charles L.: Effect of Various Types of Fluid Therapy on Survival of Monkeys Exposed to Standardized Flash Burns, Fed. Proc., 17:92, 1958 (No. 364).

7. Barnes, D. A.: Mortality of Burns at the Massachusetts General Hospital, Ann. Surg., 145:210, 1957.

8. Pillsbury, Robert D.: The Treatment of Burns, A. M. A. Arch. Indust. Health, 16:422, 1957.

9. Rosenthal, Sol Roy: The Toxin of Burns, Ann. Surg., 106:111, 1937.

10. Rosenthal, Sol Roy: Neutralization of Histamine and Burn Toxin, Ann. Surg., 106:257, 1937.

11. (a) Rosenthal, Sol Roy; Hunter, F. R.; Hunter, Alice S.; Finamore, F. J.; Williams, Florence; and Roman, Inza-Nell: Pathogenesis of Death Due to Burns. Role of the Skin, Fed. Proc., 14:124, 1955.

    (b) Rosenthal, Sol Roy; Hunter, F. R.; and Roman, Inza-Nell: On an *In Vivo* Method of Collection of Diffusates from Skin. Thermal and Radiation Injury, Arch. Intern. Pharmacodyn. (In Press).

12. (a) Rosenthal, Sol Roy; Finamore, F. J.; Hunter, F. R.; and Williams, A. D.; "Toxic Factors" in Burns, Fed. Proc., 15:156, 1956.

    (b) Rosenthal, Sol Roy: Substances Released from the Skin Following Thermal Injury: "Burn Toxin", Surgery, 46:932, 1959.

13. Rosenthal, Sol Roy, and Trahan, H: "Burn Toxin" Absorption from Burn Site, Fed. Proc., 16:370, 1957.

14. Dawe, A. R.: Combating the Effects of Burn Toxin, Naval Research Reviews, Office of Naval Research, Washington, D. C., April, 1959, p. 25.

REFERENCES (Continued)

15. (a) Naval Medical Research Unit No. 4, The Use of Tissue Cultures for the In Vitro Study of Substances in the Serum from Burned Humans, Progress Report to the Bureau of Medicine and Surgery, Navy Department, No. NM 61 01 04.1, 25 March 1959.

    (b) Rosenbaum, M. J.; Miller, L. F.; Sullivan, B.; and Rosenthal, S. R.: Inhibitory and Anti-inhibitory Factors in Acute and Healed Burn Sera by Tissue Culture Technic, Fed. Proc., 1960, and to be published.

16. Rosenthal, S. R.; Spurrier, Wilma A.; and Goodman, Alan B.: "Toxin-Antitoxin" Phenomena in Injured Animals and Man (Thermal, Radiation, and Physical Injury), Fed. Proc., 19:195, 1960, and to be published.

17. Pillmer, L.; Blum, Villa; Lipow, I. H.; Ross, D. A.; Todd, E. W.; and Wardow, A. C.: The Properdin System and Immunity, Science, 120:279, 1954.

18. Landy, M.; Skarnes, R. C.; Tropani, R. J.; and Spear, M. J.: Inactivation of Biologically Active ("Endotoxic") Polysaccharides by Fresh Human Serum, Proc. Soc. Exp. Biol. and Med., 96:744, 1957.

19. Thomas, L.: Physiopathology of the Reticulo-Endothelial System, Blackwell Pub., Oxford, 1957.

20. Ungar, G., and Damgaard, Evelyn: Protein Breakdown in Thermal Injury, Proc. Soc. Exp. Biol. and Med., 87:378, 1954.

21. Rosenthal, Sol Roy; Spurrier, Wilma A.; and Trahan, H.: Specificity of Thermal and Radiation (Beta) "Toxins" of the Skin, Fed. Proc., 17:135, 1958.

22. (a) Federov, N. A.: Experimental Immunotherapy of Burn Sickness, Proc. VI Cong. Internat. Soc. Blood Transf., No. 12:44, 1956.

    (b) Pushkar, L. W.: Demonstration of a Patient with Extensive Burn which was Treated by Immunotherapy, Terapevticheskiy Arkhiv., 2:84, (Feb.) 1958.

23. Pearson, Elinor; Soroff, H. S.; and Reiss, E.: Metabolic Derangements in Burns, Amer. Diet. Assn., 32:223, 1956.

24. Moncrief, J. A., and Rivera, J. A.: The Problem of Infection in Burns by Resistant Micro-organisms with a Note on the Use of Bacitracin, Ann. Surg., 147:295, 1958.

25. Fine, Jacob: The Bacterial Factor in Traumatic Shock, Charles C. Thomas, Springfield, Illinois, 1954.

26. Zweifach, B. W.; Bencerrai, B.; and Thomas, L.: The Relationship Between the Vascular Manifestations of Shock Produced by Endotoxin. II. The Possible Role of the Reticulo-Endothelial System in Resistance to Each Type of Shock, J. Exp. Med., 106:403, 1956.

A PROTEIN MOIETY INHIBITORY TO HeLa CELL GROWTH IN SERA OF BURNED CHILDREN

R. I. Lytle, L. F. Miller and S. R. Rosenthal (Intr. by E. R. Kirch)
Naval Medical Research Unit No. 4, Great Lakes, Illinois
and
University of Illinois College of Medicine, Chicago

Sera from acutely burned children were studied electrophoretically with a modified Aaransson technique to search for abnormal moieties not resolved by conventional methods. An abnormal component migrating more rapidly than the albumin fraction was observed when the sodium salt of ethylene-diamine tetra-acetic acid was substituted in Aaransson's buffer system at a pH of 9.1 A reduction in alpha-2 roughly equivalent to the abnormal component was noted when patterns obtained by the conventional barbital and modified techniques were compared suggesting further resolution of the alpha components with the latter. The abnormal moiety was associated with inhibition of HeLa cell growth in 17 specimens from 10 burned patients with levels of 3 to 12.5% and with absence of inhibition in 3 specimens from 3 patients with levels of 3 to 5%. The moiety was not detected in serum from unburned children. (Aided by a grant from the Office of Naval Research [NR 114 161]) (The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.)

Federation Proceedings
19:73:1960

INHIBITORY AND ANTI-INHIBITORY FACTORS IN ACUTE AND HEALED BURN SERA BY TISSUE CULTURE TECHNIC

M. J. Rosenbaum*, L. F. Miller*, B. Sullivan*, and S. R. Rosenthal
Naval Medical Research Unit No. 4, Great Lakes, Illinois
and
University of Illinois College of Medicine, Chicago

A technic is described for titrating substances in acute burn sera inhibitory to cell growth and for titrating anti-inhibitory properties of normal and healed burn sera. Varying quantities of acute burn sera were substituted for normal human serum normally used in growth of seeded HeLa cells in slanted tubes to titrate inhibitory effects on growth of the cell sheet. A box titration method was used to determine anti-inhibitory titers of normal and healed burned donor sera. Serial serum specimens taken early from severely burned children were inhibitory. Control sera did not cause inhibition. As recovery progressed inhibition diminished. Loss of inhibition was often first observed with serum collected several hours after transfusion with blood or plasma from a healed burned donor. Serum and gamma globulin from several recently healed severely burned donors had higher than normal anti-inhibitory titers. On the basis of preliminary observations, the technic is suggested as a useful *in vitro* method of employing viable cells to titrate and study the role of possible toxic-antitoxic humoral factors (in animals or man) in the pathogenesis and treatment of burns. (Aided by a grant from the Office of Naval Research [NR 114 161]).

Federation Proceedings
19:357:1960

EXPERIMENTAL BURNS IN THE GERMFREE ANIMAL

Thomas G. Ward and Louise I. Lindholm*
University of Notre Dame
Notre Dame, Indiana

To test the effect of bacteria on the shock mechanism in burns, the amount of heat expressed as LD_{50} in seconds was determined for germfree mice and for normal mice at various ages. The number of seconds required to kill half the animals when exposed by immersion in a 70°C water bath was significantly lower in the germfree mice. The amount of difference was inversely proportional to the age of the animals, germfree and normal, tested. Germfree and normal rats (Wistar) of comparable age and weight were bled, burned by a hot plate method, and sera collected at various intervals. The sera were tested for "HeLa growth preventive" factor. The normal rats responded more quickly and with a higher titer than the germfree. (Supported by a grant from Office of Naval Research.)

Federation Proceedings
19:103:1960

PROPOSAL FOR FUTURE

Studies of "Toxin-Antitoxin" Following Thermal or Traumatic Injury:

On the practical level, it is proposed to collect blood from healed burned or injured individuals and store it as whole blood, then plasma, and, finally, as the active fraction thereof. Such bloods are to be made available in the treatment of the toxicity following thermal injury (or trauma).

Eventually, it is proposed to produce an antigen from human burned skin (artificially produced *in vitro*) to use for the active immunization of those most likely to sustain such injuries and for the active immunization of animals. Such an antigen and corresponding antibodies have already been produced in animal and human studies.

On the scientific level, it is proposed to study the detailed effects of "antitoxin" administered to acutely burned patients by laboratory and clinical methods. It is further proposed to isolate and identify the "toxin" and to produce "antitoxin" artificially by animal inoculation.

To this end a center should be established which would include:

1. Hospital facilities for treatment of human burn cases.
2. Laboratories for experimental studies by *in vitro* and *in vivo* methods (tissue culture, biochemistry, serology, animal facilities).
3. Blood and skin banks with facilities for their collection, storage, and processing.

PUBLICATIONS DURING PERIOD OF REPORT

1. Graham P. Lewis, Sol Roy Rosenthal and H. Trahan (1959), "An Unidentified Smooth Muscle Stimulating Substance in Skin." Fed. Proc., 18, 91, (No. 360).

2. Sol Roy Rosenthal, Wilma A. Spurrier and Harold Trahan (1959), "A New Mouse-Scalding Technique for Long-Term Experiments with Preliminary Serologic Studies." Fed. Proc., 18 503 (No. 1982).

3. S. R. Rosenthal, L. F. Miller, J. B. Hartney, M. Rosenbaum and W. A. Spurrier (1959), "Toxin-Antitoxin Factors in Burned Human Subjects." Abstracts of Communications, XXI International Congress of Physiological Sciences, p. 237.

4. Sol Roy Rosenthal (1959), "Substances Released from the Skin Following Thermal Injury: 'Burn Toxin.'" Surgery, 46, 932.

5. R. I. Lytle, L. F. Miller and S. R. Rosenthal (1960), "A Protein Moiety Inhibitory to HeLa Cell Growth in Sera of Burned Children." Fed. Proc., 19, 73.

6. Sol Roy Rosenthal, Wilma A. Spurrier and Alan B. Goodman (1960), "'Toxin-Antitoxin' Phenomena in Injured Animals and Man (Thermal, Radiation, and Physical Injury)." Fed. Proc., 19, 195.

7. M. J. Rosenbaum, L. F. Miller, B. Sullivan and S. R. Rosenthal (1960), "Inhibitory and Anti-Inhibitory Factors in Acute and Healed Burn Sera by Tissue Culture Technic." Fed. Proc., 19, 357.

8. Sol Roy Rosenthal, F. R. Hunter, F. J. Finamore and Inza-Nell Roman (In Press), "On an <u>in Vivo</u> Method of Collection of Diffusates from Skin: Thermal and Radiation Injury." Arch. Intern. Pharmacodyn.